

COMMUNITY ASSEMBLY IN THE PRESENCE OF DISTURBANCE: A MICROCOSM EXPERIMENT

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Abstract. Ecologists know relatively little about the manner in which disturbance affects the likelihood of alternative community stable states and how the history of community assembly affects the relationship between disturbance and species diversity. Using microbial communities comprising bacterivorous ciliated protists assembled in laboratory microcosms, we experimentally investigated these questions by independently manipulating the intensity of disturbance (in the form of density-independent mortality) and community assembly history (including a control treatment with simultaneous species introduction and five sequential assembly treatments). Species diversity patterns consistent with the intermediate disturbance hypothesis emerged in the controls, as several species showed responses indicative of a trade-off between competitive ability and ability to recover from disturbance. Species diversity in communities with sequential assembly, however, generally declined with disturbance, owing to the increased extinction risk of later colonizers at the intermediate level of disturbance. Similarities among communities subjected to different assembly histories increased with disturbance, a result due possibly to increasing disturbance reducing the importance of competition and hence priority effects. This finding is most consistent with the idea that increasing disturbance tends to reduce the likelihood of alternative stable states. Collectively, these results indicate the strong interactive effects of disturbance and assembly history on the structure of ecological communities.

Key words: *alternative stable states; community assembly; disturbance; intermediate disturbance hypothesis; protists; species diversity.*

INTRODUCTION

To what extent does the structure of ecological communities depend upon their history? Despite the early recognition of the potential importance of history in shaping community structure (Gleason 1927, Egler 1954, Diamond 1975), this fundamental question remains largely unresolved. On the one hand, ample theoretical and experimental evidence suggests that multiple community configurations that differ in species composition (i.e., alternative stable states; Lewontin 1969) can arise from differences in the historical sequence of species colonization, even under identical environmental conditions and common species pools (e.g., Drake 1991, Law and Morton 1993, Blaustein and Margalit 1996, Price and Morin 2004, Fukami et al. 2007; reviewed by Samuels and Drake 1997, Chase 2003a). On the other hand, theory and experiments also show that communities with different assembly histories may nevertheless attain similar species composition and abundance, suggesting little or no role of history (e.g., Sommer 1991, Law and Morton 1996, Chase and Leibold 2003, Fukami 2004a). These conflicting patterns have led ecologists to look into factors that may

influence the role of history in community assembly, including the size of species pool (Law and Morton 1993, Chase 2003a, Fukami 2004b), productivity (Chase 2003a, b, Fukami and Morin 2003), predation (Morin 1984, Louette and De Meester 2007), ecosystem size (Drake 1991, Fukami 2004a), dispersal rate (Robinson and Edgemon 1988, Lockwood et al. 1997), and disturbance (Inouye and Tilman 1995, Chase 2003a, 2007, Trexler et al. 2005). Here we examine the effect of disturbance on the historical contingency of community assembly, a topic that has received relatively little theoretical or experimental treatment (but see Chase 2007).

Disturbance sets the stage for community assembly to occur by killing or damaging resident species and releasing resources for prospective colonizers. Furthermore, recurring disturbances that differ in intensity and/or frequency may alter community membership and species abundance, which may in turn affect the likelihood of alternative stable states (Belyea and Lancaster 1999, Chase 2003a, Didham et al. 2005, Fukami and Lee 2006). Ecologists, however, disagree on how disturbance affects the historical contingency of community assembly. Some have proposed that difference in assembly history is more likely to lead to alternative stable states in habitats experiencing low rates of disturbance (Belyea and Lancaster 1999, Chase 2003a, Fukami and Lee 2006), whereas others have

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TABLE 1. Species composition of each group used to assemble communities.

Group	Species pair
1	<i>Paramecium</i> sp., <i>Tetrahymena vorax</i>
2	<i>Paramecium aurelia</i> , <i>Blepharisma americanum</i>
3	<i>Paramecium bursaria</i> , <i>Colpidium kleini</i>
4	<i>Halteria</i> sp., <i>Spirostomum</i> sp.
5	<i>Glaucoma</i> sp., <i>Loxoecephalus</i> sp.

Note: Each of the five groups contained two species randomly selected from the 10-species pool.

proposed an increased likelihood of alternative stable states in habitats with high rates of disturbance (Didham et al. 2005, Didham and Norton 2006). Proponents of both hypotheses have drawn observational evidence to support their arguments (Chase 2003a, Didham et al. 2005). The presence of potential confounding factors in observational studies, however, precludes an unambiguous evaluation of these contrasting ideas. The only experimental work pertinent to this subject, conducted in small freshwater ponds (Chase 2007), seems to support the small likelihood of alternative stable states in disturbed habitats. However, interpretation of this experiment was clouded by the lack of control over assembly history in disturbed ponds and over the degree of environmental heterogeneity that may covary with disturbance.

A well-known aspect of disturbance is its effect on species diversity. Much research on this subject has surrounded the intermediate disturbance hypothesis (IDH), which predicts a maximum level of species diversity at intermediate levels of disturbance (Connell 1978). The IDH, however, has received mixed empirical support: besides the pattern of peaked diversity at intermediate disturbance predicted by the IDH (Shea et al. 2004), other disturbance–diversity relationships also are common (Mackey and Currie 2001). Although various hypotheses have been proposed to explain deviations from the IDH (Huston 1994, Wootton 1998, Mackey and Currie 2000, Chase and Leibold 2003), none have taken community assembly into account. The IDH often rests on the assumption that species exhibit a trade-off between competitive ability and ability to cope with disturbance (Connell 1978, Petraitis et al. 1989, Chase and Leibold 2003; but see Kadmon and Benjamini 2006). This trade-off, however, could be weakened or disrupted in communities demonstrating historical contingencies. For instance, disturbance-tolerant inferior competitors may be unable to establish in a locality where superior competitors, as early colonizers, have already built large populations. In this case, the lack of trade-off among coexisting species may produce patterns inconsistent with the IDH.

Here we report the results of a laboratory microcosm experiment that independently manipulated assembly history and disturbance intensity to examine their effects on community structure. We used fast-reproducing ciliated protists as experimental organisms, whose short

generation times enabled us to collect multigenerational data that permit more unambiguous identification of alternative stable states (Connell and Sousa 1983). The small scale of laboratory microcosms minimized spatial heterogeneity that is often present in larger systems. The experiment aimed to address two specific questions. First, how does the rate of disturbance affect the prevalence of alternative stable states associated with the historical contingency of community assembly? Second, how does the history of community assembly affect the disturbance–diversity relationship?

MATERIALS AND METHODS

The experiment used a total of 10 ciliated protist species, which were randomly assigned into five groups, each containing two species (Table 1). Most of these species were isolated from small freshwater ponds, and a few were obtained from biological supply houses. All 10 species are bacterivores that can survive on bacteria alone, although *Blepharisma americanum* and *Tetrahymena vorax* can also form carnivorous individuals that prey upon smaller conspecific and heterospecific individuals. Prior to the experiment, these species had been separately cultured in the laboratory on three bacterial species (*Bacillus cereus*, *Bacillus subtilis*, and *Serratia marcescens*) for numerous generations.

Microcosms were 250-mL Pyrex glass bottles each filled with 100 mL of medium. The medium was prepared in large Erlenmeyer flasks using a formula of 0.55 g of protozoan pellet (Carolina Biological Supply, Burlington, North Carolina, USA) per 1 L of deionized water. This medium supported the growth of bacterial food for the protists used in the experiment. Experimental containers and medium were autoclaved, after which the medium was inoculated with the bacterial assemblage present in protist stock cultures. The bacterial inoculum was prepared by mixing samples of all protist stock cultures and running the mixed sample through a 1.0- μ m sterile filter to remove the protists. The bacterized medium was distributed into individual microcosms 24 h after bacterial inoculation. Each microcosm also received two sterilized wheat seeds as an additional source of carbon.

We used a two-way factorial design with disturbance intensity and assembly sequence as the two main factors. Disturbance, which took the form of nondiscriminative density-independent mortality, had three levels: low (10% weekly mortality), intermediate (50% weekly mortality), and high (90% weekly mortality). Disturbance was imposed by sonicating different volumes of medium (low, 10 mL; intermediate, 50 mL; and high, 90 mL) in experimental microcosms using a Sonic Dismembrator Model 100 (Fisher Scientific, Waltham, Massachusetts, USA) at full power for 1 min. Microscopic inspection indicated this procedure effectively killed all living protists. The treated medium was immediately returned to its original microcosm after sonication. The assembly sequence factor had six

TABLE 2. Community assembly sequences used in the experiment.

Week	Control	Sequence 1	Sequence 2	Sequence 3	Sequence 4	Sequence 5
1	all groups	group 1	group 2	group 3	group 4	group 5
2		group 5	group 1	group 4	group 5	group 2
3		group 3	group 4	group 1	group 1	group 3
4		group 2	group 3	group 5	group 2	group 4
5		group 4	group 5	group 2	group 3	group 1

Note: See Table 1 for species composition of each group.

treatments, including a control in which all protist species were simultaneously introduced during week 1 and five sequential assembly treatments in which the five groups were sequentially introduced in a five-week span (Table 2). The inoculum of each protist species consisted of ~100 individuals. Protist stock cultures were sampled before species introduction to estimate population densities and to determine volumes of the inocula corresponding to 100 individuals. Because the age of stock cultures may affect protist species traits (e.g., body size, growth rate) and species introduction events were distributed over a relatively long period of five weeks, it was necessary to standardize the age of stock cultures upon each species introduction. Fresh stock cultures were thus established every week starting from two weeks prior to the experiment until week 3, and two-week-old stock cultures were always used to inoculate experimental microcosms.

Each treatment combination was replicated three times, resulting in 54 microcosms (3 disturbance regimes \times 6 assembly sequences \times 3 replicates). During the experiment, all microcosms were situated in a single incubator at 22°C with a 12/12 light/dark cycle. The experiment lasted 11 weeks, including 46 d after the last species introduction. Given the short generation times of the protists (*Blepharisma*, 1–2 d; others, <24 h), this period encompassed dozens of complete turnovers of even the slowest-growing community member, which was sufficient for species interactions to shape the structure of these fast-reproducing protist communities (see *Results*).

Weekly samples were taken from each microcosm to estimate the density of protist species. During sampling, ~0.4 mL of medium was withdrawn from each microcosm after mixing and distributed as multiple drops onto a preweighed petri dish; the exact mass (\approx volume) of the sample was determined using an analytic balance. The number of individuals of each protist species in the sample was enumerated under a stereoscopic microscope. To replenish nutrient and remove excessive metabolic wastes, 10 mL medium in each microcosm was replaced with fresh sterile medium weekly.

We used repeated-measures ANOVA (rm-ANOVA) to test for the effect of disturbance intensity on similarities between communities subjected to different assembly histories, measured by the Bray-Curtis similarity index (Bray and Curtis 1957). The Bray-Curtis

index ranges from 0 to 1, with increasing values indicating increased similarities among communities. Low index values, calculated for non-transient steady-state communities, would point to the possible existence of alternative stable states. In addition to similarity indices, we also used MANOVAs to directly discern whether assembly sequence and disturbance intensity affected community structure. Dependent variables in the MANOVAs were population densities of the 10 protist species at the last sampling date (week 11). We also used rm-ANOVA to assess the effects of disturbance and assembly sequence on community species richness. Within each assembly sequence, we conducted Tukey's hsd tests at each sampling date to uncover how community similarity and species richness changed with disturbance. Following Moran (2003), we did not adjust probability values of these tests using the Bonferroni procedure due to its overly conservative nature. We used ANOVAs to assess the effects of assembly sequence and disturbance on individual species densities, again using only data from the final sampling date. Data on population densities were log-transformed (\log_{10} [number of individuals/mL + 1]) before analyses.

RESULTS

Community similarity

Similarity among communities subjected to different assembly histories, represented by the Bray-Curtis index, differed significantly among disturbance levels (Fig. 1; rm-ANOVA, $F = 13.94$, $df = 2, 402$, $P < 0.0001$). This difference was mainly caused by significantly larger similarity values at higher rates of disturbance during early weeks (Fig. 1; Tukey's hsd tests). Community similarity showed different temporal patterns under different disturbance intensities: it increased over time under low disturbance, first increased then decreased under intermediate disturbance, and remained about the same level under high disturbance (Fig. 1), resulting in a significant time effect ($F = 20.67$, $df = 6, 397$, $P < 0.0001$) and a significant disturbance \times time effect ($F = 10.28$, $df = 12, 794$, $P < 0.0001$) in the rm-ANOVA. By the end of the experiment, community similarities at the low and high levels of disturbances were similar, whereas similarity at the intermediate level of disturbance was significantly lower (Fig. 1; Tukey's hsd test). The decline in similarity under intermediate disturbance was largely due to the difference between the controls and sequential assembly treatments that grew greater during the later

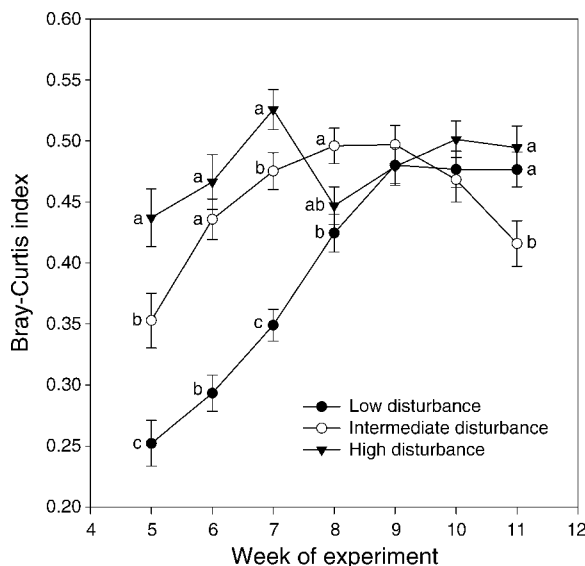


FIG. 1. Similarity (measured by the Bray-Curtis index) among communities subjected to different assembly regimes at each of the three disturbance levels through time. Mean similarity was obtained by averaging index values from all possible pairwise comparisons of communities. Each community consisted of 10 ciliated protist species, assigned to five groups (each containing two species; see Tables 1 and 2) with different assembly sequences of the groups. Communities experienced three levels of disturbance (low, 10% weekly mortality; intermediate, 50% weekly mortality; and high, 90% weekly mortality). Different lowercase letters associated with symbols represent significant differences at $P = 0.05$ in a Tukey's hsd test conducted for each sampling date; insignificant differences are not shown. Values are means \pm SE.

stage of the experiment. When only the five sequential assembly treatments were used for similarity index calculations, no significant temporal decline in community similarity was observed under intermediate disturbance and similarity for the three levels of disturbance eventually converged (results not shown). Despite this convergence in community similarity, MANOVA on species abundances at the last sampling date revealed a significant disturbance \times sequence effect ($F = 1.49$, $df = 80$, 192.15 , $P = 0.0145$), suggesting differential effects of history under different levels of disturbance. MANOVA conducted at each disturbance level revealed highly significant differences under low disturbance ($F = 3.50$, $df = 35$, 27.67 , $P = 0.0006$), significant differences under high disturbance ($F = 1.86$, $df = 30$, 30 , $P = 0.0471$), and nonsignificant differences under intermediate disturbance ($F = 1.29$, $df = 40$, 20.589 , $P = 0.2547$). When the controls were excluded from MANOVA, there were highly significant differences under low disturbance ($F = 3.71$, $df = 28$, 15.844 , $P = 0.0042$), significant differences under intermediate disturbance ($F = 2.47$, $df = 32$, 12.659 , $P = 0.0445$), and nonsignificant differences under high disturbance ($F = 1.73$, $df = 24$, 18.653 , $P = 0.1159$). Thus both similarity indices and MANOVA indicated

that communities with different assembly histories became more similar with increasing disturbance.

Species richness

Species richness declined over time in all microcosms (rm-ANOVA, time, $F = 48.12$, $df = 6$, 31 , $P < 0.0001$; Fig. 2), and the tempo of decline varied with assembly sequence (sequence \times time, $F = 2.15$, $df = 30$, 126 , $P = 0.0017$) and disturbance intensity (disturbance \times time, $F = 5.65$, $df = 12$, 62 , $P < 0.0001$). Both assembly sequence ($F = 28.52$, $df = 2$, 36 , $P < 0.0001$) and disturbance ($F = 4.61$, $df = 5$, 36 , $P = 0.0024$) had significant main effects on species richness, with no significant interactions between the two factors ($F = 1.30$, $df = 10$, 36 , $P = 0.2661$). While the effects of assembly sequence appeared to be idiosyncratic, increasing disturbance, in general, tended to reduce species richness (Fig. 2). Despite this average negative effect of disturbance and the nonsignificant sequence \times disturbance term in the rm-ANOVA, richness patterns in the controls differed markedly from the five sequential assembly treatments. In the controls, richness under intermediate disturbance was significantly higher than those under low and high disturbances during weeks 8 and 9 and was significantly higher than those under high but not low disturbances during the following weeks (Fig. 2A; Tukey's hsd tests). In the five sequential assembly treatments, however, richness never peaked at intermediate disturbance and generally declined with disturbance (Fig. 2B–F).

Individual species patterns

Responses to experimental treatments differed considerably among species. *Tetrahymena vorax* and *Halteria* went extinct during early stages of the experiment in all microcosms, regardless of the level of disturbance or the history of community assembly (Appendix A: Figs. A1–A6). By contrast, *Colpidium* persisted at appreciable densities in all treatments (Fig. 3; Appendix A: Figs. A1–A6), and its final density was significantly affected by assembly sequence but not disturbance (Appendix B: Table B1). Densities of the other species were all affected by disturbance (all P 's < 0.05 ; Appendix B: Table B1), though their responses to disturbance differed. Many species declined in abundance with increasing disturbance. For instance, *Paramecium bursaria* persisted in all microcosms under low disturbance, persisted in some microcosms under intermediate disturbance, but went extinct in all microcosms under high disturbance (Fig. 3; Appendix A: Figs. A1–A6). *Blepharisma* show extremely similar patterns to *P. bursaria*, except it was present in one high-disturbance microcosm at the end of the experiment. By contrast, some species, including *Loxocephalus* and *Paramecium aurelia*, appeared to have benefited from disturbance. *Loxocephalus* was unable to persist in low-disturbance environments; however, it was able to sustain its populations in at least some replicates with

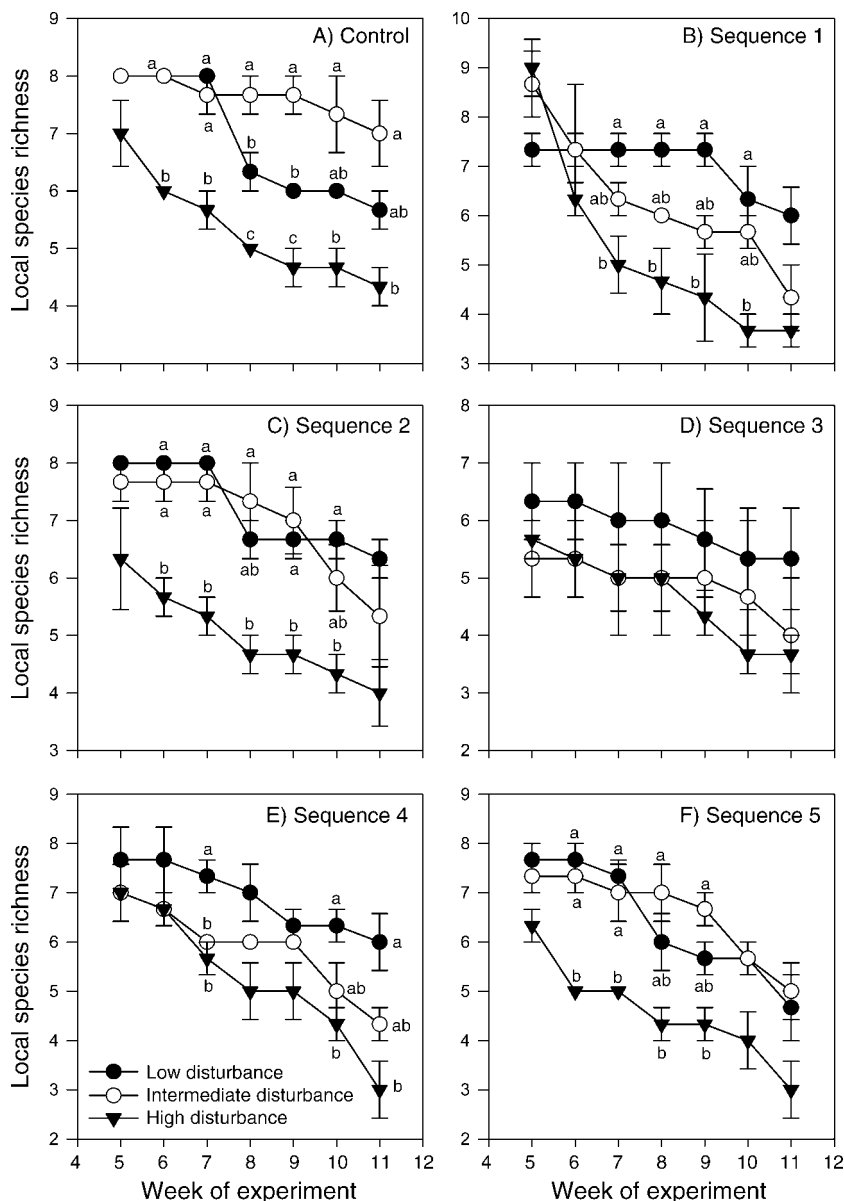


FIG. 2. Species richness at each of three disturbance levels through time for the control and sequences 1–5. Each community consisted of 10 ciliated protist species, assigned to five groups (each containing two species; see Tables 1 and 2) with different assembly sequences of the groups. Communities experienced three levels of disturbance (low, 10% weekly mortality; intermediate, 50% weekly mortality; and high, 90% weekly mortality). Different lowercase letters represent significant differences at $P=0.05$ in a Tukey's hsd test conducted for each sampling date; insignificant differences are not shown. Values are means \pm SE.

increased rate of disturbances, especially in the high-disturbance environments (Fig. 2; Appendix A: Figs. A1–A6). Similar pattern held for *P. aurelia*, although it was only completely eliminated under low disturbance in the controls (Fig. 3; Appendix A: Figs. A1–A6).

DISCUSSION

There has been much recent debate regarding how disturbance affects the likelihood of alternative stable states in community structure (Chase 2003a, Didham et al. 2005, Didham and Norton 2006, 2007, Fukami and

Lee 2006, Mason et al. 2007). This debate, however, has been largely based on discussions of conceptual models and observational evidence, and more objective evaluations of these ideas using mathematical models or manipulative experiments have been scarce (but see Chase 2007). Here we provide a direct experimental test of how disturbance affects the possibility of alternative stable states associated with different assembly histories. Similarity among communities subjected to different assembly histories was higher in microcosms experiencing higher rates of disturbance. This is most consistent

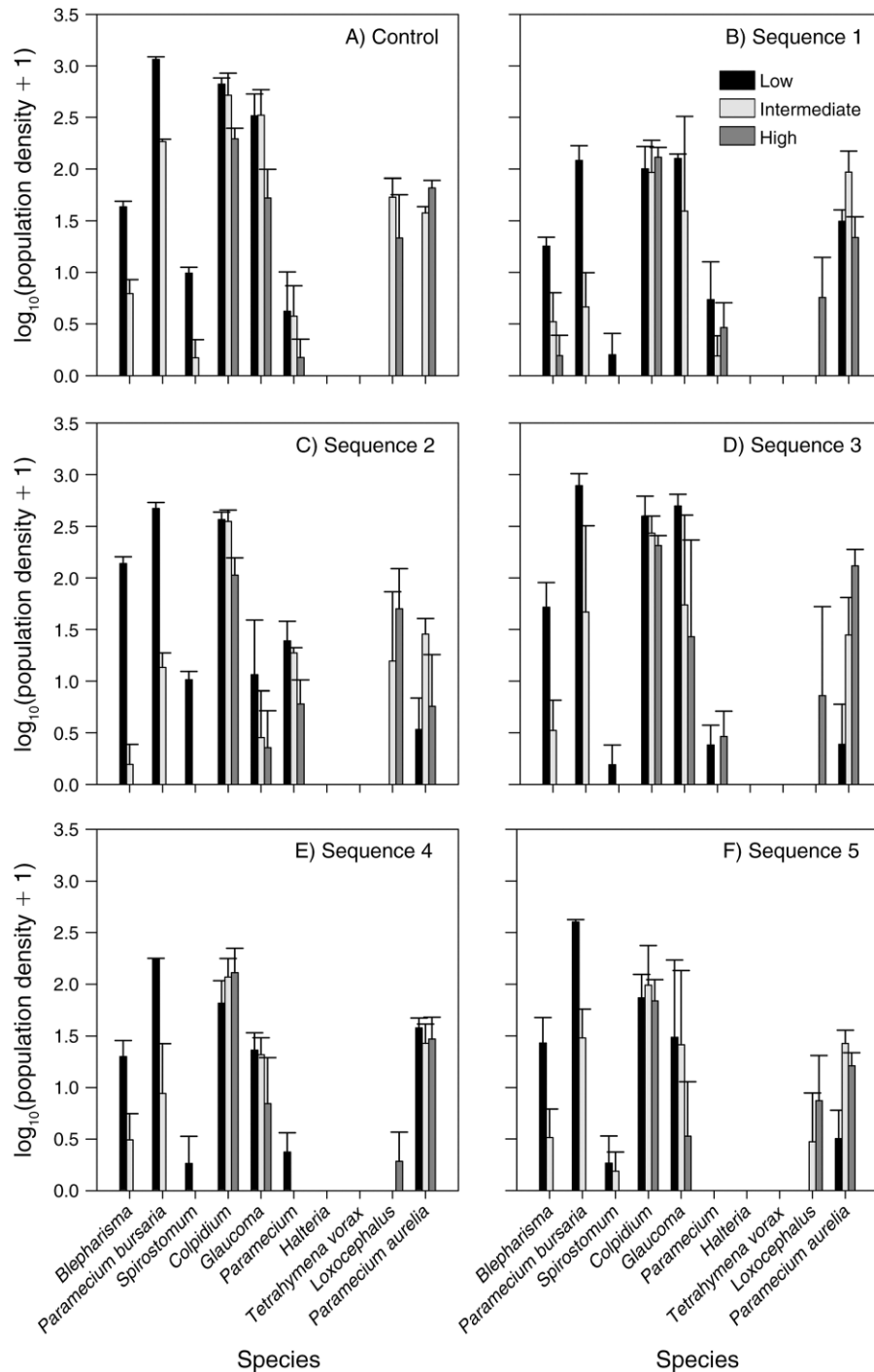


FIG. 3. Population density of each protist species at each of the three disturbance levels on the final sampling date (week 11) for the control and sequences 1–5. Values are means + SE with density data originally measured as no. individuals/mL and log₁₀-transformed prior to analyses.

with the idea that alternative community stable states are more likely to arise from different assembly histories in environments characterized by lower rates of disturbance than by higher rates of disturbance (Belyea and Lancaster 1999, Chase 2003a, Fukami and Lee 2006). In

an experiment conducted in small freshwater ponds, Chase (2007) also showed that similarity was greater among pond communities experiencing drought than those lacking drought disturbance. Several observational studies also reported findings consistent with this idea.

For instance, Inouye and Tilman (1995) examined similarity in plant community structure in several old fields and in a savanna with no historical agricultural practice. They found that communities within each of the two recently disturbed old fields were more similar, whereas communities that were in much older old fields (i.e., longer time since disturbance) and native savanna were less similar. Likewise, Chase (2003a) compared animal species composition in freshwater ponds classified into different permanence categories (characterized by different drying [disturbance] frequencies) and found that similarity among pond communities increased as ponds became less permanent (i.e., more frequent drying). Trexler et al. (2005) obtained essentially the same results when examining the effect of drying frequency on small fish communities in the Everglades.

Given that communities at the end of our experiment did not appear to have settled into distinct states characterized by different species composition (Appendix A: Figs. A1–A6), one could argue that increased community similarity under a higher rate of disturbance may not directly translate into the reduced likelihood of alternative stable states under a higher rate of disturbance. Our experiment, however, may have yet reached steady states. This is despite the fact that its duration was sufficient for dozens of complete community turnovers, longer than those of most existing experiments on alternative stable states (reviewed by Schroder et al. 2005). Nevertheless, the structure of communities going through transient phases may simply differ as the result of community divergence towards alternative stable states, barring any stochastic events that can lead to unexpected divergence in community structure (random divergence, *sensu* Schroder et al. 2005). Thus differences in transient community patterns could be indicative of potential differences in alternative community stable states (Didham et al. 2005). Indeed, divergence in species composition occurred in the absence of disturbance at the end of a 15-week assembly experiment that used the same set of protist species, albeit a different set of colonization sequences (L. Jiang and Z. Pu, *unpublished manuscript*). Moreover, even if communities may eventually converge to similar structures in the absence of alternative stable states, the presence of possible long-term transients in many systems (Hastings 2004) suggests the need to understand the role of community assembly for long-term transient dynamics (Fukami 2004a, Schroder et al. 2005). Fukami (2004a) has experimentally demonstrated that assembly history can affect species diversity for long periods of time before its effect disappears. Our finding would be qualitatively similar to that of Fukami (2004a) should community convergence occur. Overall, our results indicate that disturbance can interact with assembly history to affect community structure for an ecologically meaningful time span, regardless of whether observed patterns represent transient or steady states.

Why were communities with different assembly sequences more similar with increasing disturbance? The answer seems to lie in the fact that competition became less important in habitats characterized by high disturbance rates, an idea first proposed by Grime (1979). Recall that individual species responses to disturbance were consistent with a trade-off between species' ability to compete and their ability to cope with disturbance (Fig. 3). Increasing disturbance caused density reduction and in many cases (especially under high disturbance) extinction of disturbance-intolerant species that also appeared competitively superior. Consequently, communities under higher disturbance were increasingly characterized by disturbance-tolerant species that also appeared to be inferior competitors. The reduced role of competition means that early colonizing species had lesser effects on species that arrive later and were less likely to preclude late-arriving species, which should increase community similarity and reduce the likelihood of alternative stable states (Chase 2003a, Fukami and Lee 2006). To directly examine the manner in which competition varied with disturbance, we conducted multiple regressions within each disturbance level to relate the density of each of the four common species (*Colpidium*, *Loxocephalus*, *P. aurelia*, and *Glaucoma* sp.) characteristic of high-disturbance environments to densities of all its potential competitors. Only data from the final sampling date were used in multiple regressions, which employed the backward elimination procedure and a significance level of 0.05. Regressions revealed that *Colpidium* was not significantly adversely affected by other species at all disturbance levels; that *Loxocephalus* was not significantly adversely affected by other species at intermediate and high levels of disturbance (*Loxocephalus* was driven to extinction everywhere in low-disturbance microcosms); that *Glaucoma* was adversely affected by *P. aurelia* at the low level of disturbance only; and that *P. aurelia* was adversely affected by *P. bursaria* at low and intermediate levels of disturbance only (Fig. 4). In addition, the per capita (i.e., the slope of the regression) and overall (i.e., per capita \times *P. bursaria* density) negative effects of *P. bursaria* on *P. aurelia* were greater at the low than at the intermediate level of disturbance (Fig. 4). These results thus provide suggestive, though not confirmatory, evidence that the strength of competition may have declined with increasing disturbance.

Our results did not lend much support to the view that disturbance promotes alternative stable states (Didham et al. 2005, Didham and Norton 2006). Didham and colleagues (Didham et al. 2005, Didham and Norton 2006) argue that because species traits in habitats characterized by high rates of disturbance are more similar than those in habitats characterized by low rates of disturbance, early colonizers are more likely to resist invasion of later colonizers that share more similar traits, resulting in higher probability of alternative stable states with increasing disturbance. With the increasing

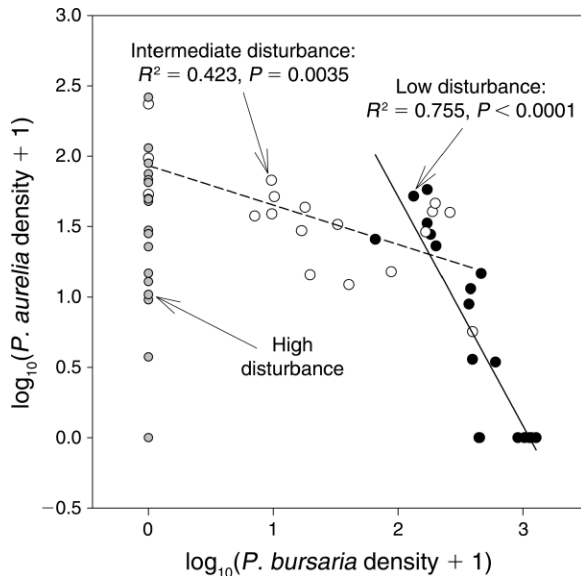


FIG. 4. *Paramecium aurelia* density as a function of *P. bursaria* density at each of the three disturbance levels on the final sampling date (week 11). Symbols: black circles and solid regression line, low disturbance; open circles and dashed regression line, intermediate disturbance; gray circles, high disturbance. Density data, originally measured as no. individuals/mL, were \log_{10} -transformed prior to analyses.

commonness of disturbance-tolerant species under higher disturbance, species traits in habitats with higher rates of disturbance did become more similar in our experiment. However, the increased similarity in species traits is not necessarily equivalent to increased competition among community members. In our experiment, competition appeared to have weakened in habitats with high disturbance despite the increase in species similarity (see the previous paragraph). As suggested by Chase (2003a) and Fukami and Lee (2006) and supported by our experiment results, the more likely outcome of this reduced role of competition in high disturbance habitats is increased similarity among communities with different assembly sequences and small likelihood of alternative stable states.

Species diversity pattern consistent with the IDH was found only in the controls with simultaneous species colonization, but not in the five sequential assembly treatments comprising multiple species introduction events. The deviation from the IDH in most assembly treatments accords with the general finding that only a small fraction of empirical studies testing the IDH actually supported it (Mackey and Currie 2001). A number of factors, such as environmental productivity (Huston 1979, Kondoh 2001, Scholes et al. 2005), predation (Kneitel and Chase 2004, Morgan and Buckling 2004, Gallet et al. 2007), trophic positions of study organisms (Wootton 1998), and the spatial scale at which disturbance occurs (Chase 2003a, Chase and Leibold 2003), are known to influence the relationship

between disturbance and diversity, and deviations from the IDH could potentially arise from variations in any of these factors. The different diversity responses to disturbance in the controls and sequential assembly treatments suggest that the history of community assembly should be incorporated into the suite of factors impacting the diversity–disturbance relationship.

The IDH often assumes a trade-off between competitive ability and ability to cope with disturbance (Connell 1978, Petraitis et al. 1989, Chase and Leibold 2003), and results in the controls were consistent with the role of this trade-off in producing the unimodal diversity–disturbance relationship. In the controls, *Blepharisma* and *P. bursaria*, presumably strong competitors, dominated under low disturbance but were driven to extinction under high disturbance; *Loxocephalus* and *P. aurelia*, presumably weak competitors, were driven to extinction under low disturbance but were common under high disturbance; only at the intermediate level of disturbance did both types coexist (Fig. 3A; Appendix A: Fig. A1). The lack of support for the IDH in the sequential assembly treatments may be explained by the elevated extinction rates of species at both ends of the trade-off under intermediate disturbance. This resulted because later colonizers, especially those at the ends of the competition–tolerance axis, generally had difficulty building viable populations under intermediate disturbance. *Loxocephalus*, a “tolerant” species, almost always failed to establish under intermediate disturbance if it was not the first to colonize habitats (Appendix A: Figs. A1–A6); competition from early colonizers presumably drove it to extinction. Competitive superiors, such as *Blepharisma* and *P. bursaria*, also frequently experienced extinction at the intermediate level of disturbance under sequential assembly (Appendix A: Figs. A1–A6). These disturbance-intolerant superior competitors were more vulnerable to disturbance when introduced later, as competition from early colonizers prevented them from building sufficiently large populations to endure disturbance of the intermediate intensity. Note that this absence of species at both ends of the trade-off did not occur at low and high levels of disturbance, where competitively superior (disturbance-intolerant) and inferior (disturbance-tolerant) species dominated, respectively. Using a similar experimental system, Cadotte (2007) also showed that the lack of competition–colonization trade-off among species, another trade-off frequently invoked to explain the IDH, may cause species diversity to decline with disturbance. The current study and that of Cadotte (2007) thus support the importance of ecological trade-offs for producing the unimodal diversity–disturbance relationship (but see Kadmon and Benjamini 2006). Our experiment further suggests that the lack of competition–tolerance trade-off associated with sequential community assembly may be a likely explanation behind the low occurrence of IDH patterns in empirical studies, since different species

generally colonize natural communities sequentially rather than simultaneously.

Several caveats may potentially limit the generality of our results. First, our experiment was conducted in laboratory microcosms using species that may or may not co-occur in nature. Based on this fact, one could argue that our findings may not be directly generalized to natural systems. The use of laboratory microcosms and protists with simple life histories, however, allowed us to examine relatively long-term community dynamics that otherwise would not be possible with most natural systems. The use of microcosms also minimized the presence of environmental heterogeneity, which could covary with disturbance in larger systems (e.g., Chase 2007). Second, in our experiment disturbance was implemented using sonication, which mimicked nonselective density-independent mortality that killed all individuals affected by disturbance. While density-independent mortality is not uncommon in nature (Huston 1994), it remains to be seen whether our results can apply to other types of mortality associated with different disturbance regimes (e.g., density-, size-, or species-dependent mortality, *sensu* Huston 1994). Third, species may tolerate disturbance by being either resistant during disturbance and/or being resilient after disturbance. The nondiscriminative nature of our disturbance regimes, however, suggests that here species tolerance is being measured by only resilience and that the competition–tolerance trade-off is essentially a competition–resilience trade-off. Future experiments with different disturbance regimes are necessary to test whether competition–resistance trade-offs could produce similar results.

Despite these caveats, our results clearly demonstrate that disturbance and community assembly interact to affect community structure. Increasing disturbance led to an increase in the similarity among communities subjected to different assembly sequences, suggesting that alternative stable states are less likely in habitats characterized by high rates of disturbance. Community assembly history had a major impact on the relationship between disturbance and species diversity, with patterns consistent with the IDH observed only for communities assembled via a single colonization event, but not for communities assembled via multiple sequential species colonization events. Although much attention has been given to the ecological consequences of disturbance and community assembly, few studies have examined the combined effects of both factors. The strong interactive effects of disturbance and assembly history found in this study suggest that further understanding of their ecological roles may be attained by considering both factors simultaneously.

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APPENDIX A

Protist population dynamics in different treatments (*Ecological Archives* E089-112-A1).

APPENDIX B

Results of ANOVAs for the effects of disturbance and assembly sequence on protist abundances (*Ecological Archives* E089-112-A2).